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Proceedings of the National Academy of Sciences of the United States of America, Vol. 85, No. 23 (Dec. 1, 1988), 8910-8913.

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Tue Dec 16 12:19:16 2003

Human DNA topoisomerase I is encoded by a single-copy gene that maps to chromosome region 20q12-13.2

(human *TOP1* gene/molecular cloning/*in situ* hybridization/mouse-human hybrids)

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Contributed by James C. Wang, August 24, 1988

ABSTRACT cDNA clones of the human *TOP1* gene encoding DNA topoisomerase I (EC 5.99.1.2) have been obtained by immunochemical screening of phage λ libraries expressing human cDNA segments, using rabbit antibodies raised against purified HeLa DNA topoisomerase I. Hybridization patterns between the cloned cDNA sequences and human cellular DNA and cytoplasmic mRNAs indicate that human *TOP1* is a single-copy gene. The chromosomal location of the gene has been mapped to the long arm of chromosome 20, in the region q12-13.2, by hybridization of a radioactively labeled *TOP1* cDNA probe to human metaphase chromosomes and to a panel of rodent-human somatic hybrids retaining overlapping subsets of human chromosomes.

Eukaryotic DNA topoisomerase I (EC 5.99.1.2) was identified in extracts of mouse cells in 1972 (1) and has since been found in all eukaryotes (for recent reviews, see refs. 2–4). The enzyme catalyzes interconversions between different topological forms of DNA by transiently breaking DNA strands one at a time; it is therefore classified as a type I DNA topoisomerase (5).

It has been known for many years that the eukaryotic enzyme is distinct from prokaryotic DNA topoisomerase I in terms of its substrate specificity and mechanism of catalysis. Recent sequencing of the *TOP1* gene encoding the enzyme in two distantly related yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (6, 7), shows that the primary structure of the enzyme from the two organisms is closely related; sequence similarity is also found between the yeast enzymes and a type I DNA topoisomerase encoded by vaccinia virus (8). When the amino acid sequence of *S. cerevisiae* topoisomerase I is compared with that of *Escherichia coli* DNA topoisomerase I, however, no significant similarity is found (9). In spite of this lack of similarity, the expression of active yeast DNA topoisomerase I in *E. coli* has been shown to functionally complement the lethal phenotype of a mutation in the *topA* gene encoding the *E. coli* enzyme (10). These results indicate that the biological functions of eukaryotic and bacterial DNA topoisomerase I are a manifestation of their common denominator, namely, their catalysis of DNA topoisomerization.

Eukaryotic DNA topoisomerase I appears to participate in a number of vital cellular processes including replication and transcription (2–4; 11–14). Yeast *top1* mutants, including deletion mutants, are nevertheless viable. Studies with yeast *top1 top2* double mutants indicate that the nonessentiality of DNA topoisomerase I is likely to be due to the substitution

of the enzyme by DNA topoisomerase II (EC 5.99.1.3), a type II topoisomerase (15).

Recent studies identify the DNA topoisomerases as the targets of a number of anticancer drugs (16–20). Whereas the majority of these therapeutics act on DNA topoisomerase II, one, the plant alkaloid camptothecin, acts on DNA topoisomerase I (21–25). The biological and clinical importance of DNA topoisomerases prompted us to embark on the identification of the human genes encoding these enzymes. In a recent communication, we have reported results on the cloning, sequencing, and chromosomal location of the human *TOP2* gene encoding DNA topoisomerase II (26); we report here parallel studies on the molecular cloning and mapping of the chromosomal location of the human *TOP1* gene. D'Arpa *et al.* (27) have recently reported their work on the identification and nucleotide sequencing of cDNA clones of human *TOP1*.

MATERIALS AND METHODS

Materials. HeLa DNA topoisomerase I and rabbit antisera specific to the enzyme were prepared as described (14). A HeLa cDNA library in phage λ gt10 was kindly provided by R. Tjian (University of California at Berkeley). Isolation, propagation, and characterization of human and mouse parental cell lines and somatic cell hybrids used in this work as well as the extraction of DNA from these cells were done as described previously (28, 29). Peripheral blood from healthy volunteers was used in the preparation of metaphase chromosomes. All other materials were obtained from commercial sources.

Methods. Immunochemical screening for the expression of human DNA topoisomerase I antigenic determinants in phage λ -infected *E. coli* cells was carried out according to the method of Young and Davis (30). Plaque hybridization, subcloning of the human DNA inserts in the phage λ vector, nucleotide sequencing of the subclones by the Sanger dideoxynucleotide chain-termination method, and hybridization of blots of agarose gels were carried out according to standard procedures. Expression and purification of a protein fragment encoded by the *EcoRI* human cDNA segment D1 in the clone λ h*TOP1*-D1 for raising rabbit antibodies against human DNA topoisomerase I was done in a phage T7 expression system (31). *In situ* hybridization of 3 H-labeled DNA fragment to metaphase chromosomes was done according to the procedures of Harper and Saunders (32).

RESULTS

Molecular Cloning of cDNA Encoding Human DNA Topoisomerase I. A human HepG2 cDNA library in a λ gt11 expression vector (30) was used to transform *E. coli* cells, and

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transformants were screened immunochemically for the expression of antigenic determinants that are recognized by rabbit antibodies raised against purified HeLa DNA topoisomerase I. A positive clone, λ hTOP1-D1, containing a human DNA insert of 704 base pairs was identified. Nucleotide sequencing of the insert following its subcloning was carried out, and the sequence obtained identifies an open reading frame spanning the entire insert. The amino acid sequence deduced from it shows that 50% of the amino acids are identical to those at the corresponding positions between Glu-137 and Lys-374 of *S. cerevisiae* DNA topoisomerase I (6). A comparable degree of sequence similarity was also observed between the deduced sequence and that of *Schizosaccharomyces pombe* DNA topoisomerase I between Glu-185 and Lys-419 (7). When the human DNA insert in the clone was expressed in *E. coli* from a phage T7 promoter (31) and the product was purified and used to elicit rabbit antibodies, the antibodies were found to bind specifically to human DNA topoisomerase I purified from HeLa cells and also to a 100-kDa protein band in whole cell extracts. Furthermore, in extracts of HeLa cells that had been treated with camptothecin, which is known to specifically deplete cellular DNA topoisomerase I by trapping the enzyme to DNA (21, 22), the 100-kDa protein band became much less prominent upon immunostaining with antibodies against the TOP1-D1-encoded peptic fragment (data not shown). These results provided strong evidence that the cloned segment is part of the coding segments of human TOP1 gene.

The human DNA insert D1 from λ hTOP1-D1 was used as the probe in subsequent screens of a HeLa cell cDNA library in λ gt10. Several clones were identified; partial sequencing of subclones of the human DNA insert in one of them, denoted λ hTOP1-D2, indicates that this clone contains a 2.7-kilobase pair human DNA insert that encodes a polypeptide chain homologous to the carboxyl two-thirds of yeast DNA topoisomerase I, plus 1.0 kilobase (kb) of untranslated sequence on the 3' side. There is a 469-base-pair overlap between the 3' end of the human DNA insert in λ hTOP1-D1 and the 5' end of the human DNA insert in λ hTOP1-D2. The beginning 39 nucleotides of the 5' end of the latter, however, do not match the corresponding nucleotides in the former. Thus sequence rearrangement has apparently occurred during the construction of the particular HeLa cDNA library or its subsequent passage. Such rearrangements were also observed in several of the human DNA topoisomerase II cDNA clones from the same library (22).

Human DNA Topoisomerase I Is Encoded by a Single-Copy Gene. Hybridization of HeLa cytoplasmic mRNAs, which had been resolved by electrophoresis in a formaldehyde gel, revealed that a single 4000-nucleotide RNA band was recognized by the 32 P-labeled human DNA insert from λ hTOP1-D1 (Fig. 1). Hybridization of several restriction enzyme digests of human cellular DNA with labeled TOP1 cDNA clones, either the 0.7-kb D1 insert in λ hTOP1-D1 or the 1.8-kb *Eco*RI insert in λ hTOP1-D2, all gave simple patterns that are consistent with human TOP1 gene being a single-copy gene (results not shown). This conclusion is strengthened further by the mapping of the gene to a single locus on the long arm of chromosome 20, as described in the sections below.

The Human TOP1 Gene Is Located on Chromosome 20. To determine the chromosomal location of the human TOP1 gene, a panel of 20 well-characterized rodent-human somatic cell hybrids retaining overlapping subsets of human chromosomes or chromosome regions were tested for their retention of the gene by blot hybridization (33). Cellular DNA was prepared from each of the lines and digested with the *Eco*RI restriction endonuclease to completion. After electrophoresis in a 0.8% agarose gel and transfer of the resolved DNA fragments to a nylon sheet, hybridization of the nylon-bound DNA was carried out with 32 P-labeled D1 DNA. An example

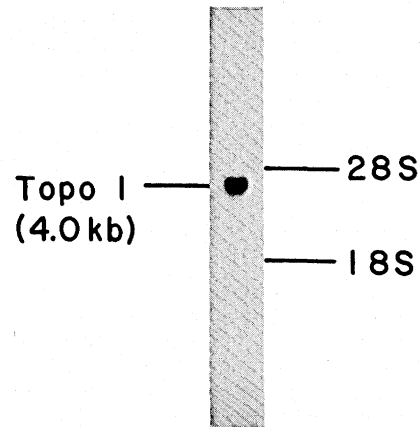


FIG. 1. A single 4.0-kb band was revealed by hybridization between human cytoplasmic mRNAs purified by chromatography on an oligo(dT) column and 32 P-labeled RNA transcribed from the human TOP1 fragment D1 subcloned into vector pGEM4 (Promega Biotec, Madison, WI). The lines 28S and 18S mark the positions of the two ribosomal RNAs.

of such a blot is depicted in Fig. 2. Murine DNA contains two *Eco*RI fragments, 7.2 and 4.0 kb in length, that are recognized

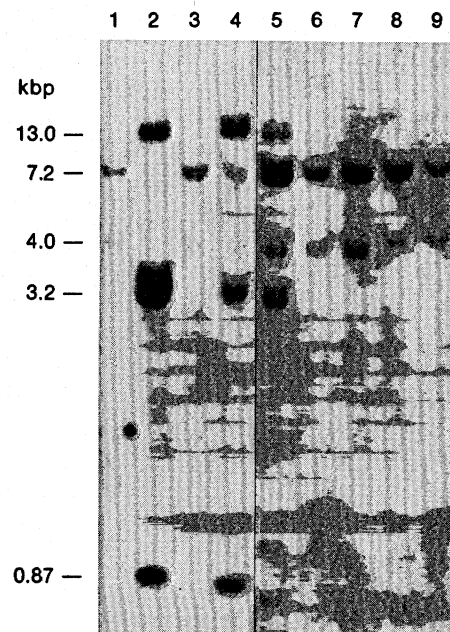


FIG. 2. Localization of human TOP1 gene to chromosome 20 by hybridization of the cloned D1 insert to DNA samples from rodent-human somatic hybrids retaining overlapping sets of human chromosomes or chromosome regions. Each lane contained $\approx 10 \mu\text{g}$ of *Eco*RI-digested DNA from the following sources: mouse (lane 1); human T-cell line (lane 2); hybrid 8c retaining human chromosomes 4, 9, 12, 17, 21, 22, and parts of chromosomes 6 and 8 (lane 3); hybrid 77-31 retaining human chromosomes 1, 3, 5-9, 13, 14, 17, 20, 22, and X and parts of chromosomes 4 and 10 (lane 4); hybrid AB3 retaining human chromosomes 6 and X, 20pter-20q13, and part of chromosome 14 (lane 5); hybrid S3 retaining human chromosomes 3, 6, 9, 14, 15, 17, 22, and X (lane 6); hybrid EF3 retaining human chromosomes 7, 14, 19, 21, and X and parts of chromosomes 8 and 22 (lane 7); hybrid 2S5 retaining parts of human chromosomes 4, 6, 12, 13 and 14 (lane 8); hybrid c131 retaining human chromosome 17 (lane 9). Electrophoresis in a 0.8% agarose gel, blot-transfer of the gel-resolved DNA fragments to a nylon membrane, and hybridization between the membrane-bound DNA and radiolabeled human TOP1 cDNA D1 fragment were carried out by standard procedures. Molecular lengths of the *Eco*RI fragments of the human and murine TOP1 genes that are specifically recognized by the D1 probe are indicated (in kb) at left.

by the D1 probe (Fig. 2, lane 1). Human DNA contains four *Eco*RI fragments that are recognized by the D1 probe: fragments that are 13, 3.2, and 0.87 kb long can be seen in lane 2 of Fig. 2; a smaller fragment of <0.5 kb in length had run off the gel and is therefore absent in the particular autoradiogram. For the DNA samples from the somatic hybrids analyzed in lanes 3–9 of Fig. 2, only two (lanes 4 and 5) exhibited the human *TOP1*-specific fragments.

Results of analysis of DNAs from the entire panel of 20 rodent–human hybrids for the presence of the human *TOP1* gene are summarized in Fig. 3, where the presence or absence of the gene in a hybrid is indicated in the column to the right of the figure by a plus or minus sign. Inspection of Fig. 3 reveals that the presence of the human *TOP1* gene correlates perfectly with the presence of human chromosome 20 and not with any other human chromosomes. Furthermore, the presence of the human *TOP1* sequence in hybrid AB3, which retains only the region 20pter–20q13 of human chromosome 20 (29), shows that the gene is contained in this region of the chromosome.

In Situ Hybridization of Cloned Human *TOP1* cDNA to Metaphase Chromosomes Locates the Gene to Chromosome Region 20q12–13.2. The location of the human *TOP1* gene was also determined by *in situ* hybridization to metaphase chromosomes by using a ³H-labeled human cDNA insert from *hTOP1*-D1 as the probe.

As summarized in Fig. 4, out of a total of 113 grains from 40 metaphases, 22 (20%) were located on the long arm of chromosome 20 (20q); 86% of these grains were clustered around a single band at the level of resolution in our preparations. This band, which was designated q12 in earlier years, has more recently been split into two subregions and is designated 20q12–13.2 according to the 1985 revision of the International System for Human Cytogenetic Nomenclature (34). An increase in the number of grains over the background was also observed at chromosome 2 in the q34 region; the increment is not statistically significant, however.

DISCUSSION

We have obtained cDNA clones of human DNA topoisomerase I and used the cloned DNA fragments to show that the human *TOP1* gene is a single-copy gene located on the long arm of chromosome 20 in the region q12–13.2. Chromosome 20 is not yet saturated with DNA markers, and the *TOP1* gene should provide a useful marker. As in the case of other organisms that have been examined (2–4, 15), the two known human DNA topoisomerase genes *TOP1* and *TOP2* are located on different chromosomes.

Two other groups have recently obtained cDNA clones of human DNA topoisomerase I independently (refs. 27 and 36). In the work of D'Arpa *et al.* (27), autoantibodies from scleroderma patients were used in the immunoprobings of human DNA topoisomerase I, whereas in our work rabbit antibodies elicited by purified HeLa DNA topoisomerase I were used. Our cloning and nucleotide sequencing results are in agreement with the published data (27): the nucleotide sequence of the 704-base-pair D1 clone of this work is identical to base pairs 624–1327 of the reported sequence; the size of human *TOP1* mRNA reported here, 4000 nucleotides, is the same as that reported, 4100 nucleotides, within experimental error. D'Arpa *et al.* (27) have also shown that human *TOP1* is most likely a single-copy gene.

A 1.1-kb 3' untranslated region is present in both of the cDNA clones D2 (this work) and T1A (27). If the 3' ends of these clones are close to the end of the human *TOP1* message, it could be readily estimated from the size of the mRNA and the length of the coding sequences that there might be a 5' untranslated region ≈700 nucleotides in length. Identification of the transcriptional start site and sites that might affect the expression of the gene awaits further studies.

As mentioned in the introductory section, human DNA topoisomerase I has recently been shown to be the intracellular target of a cytotoxic drug camptothecin (21–25). Whereas the antitumor activities of the drug against a broad

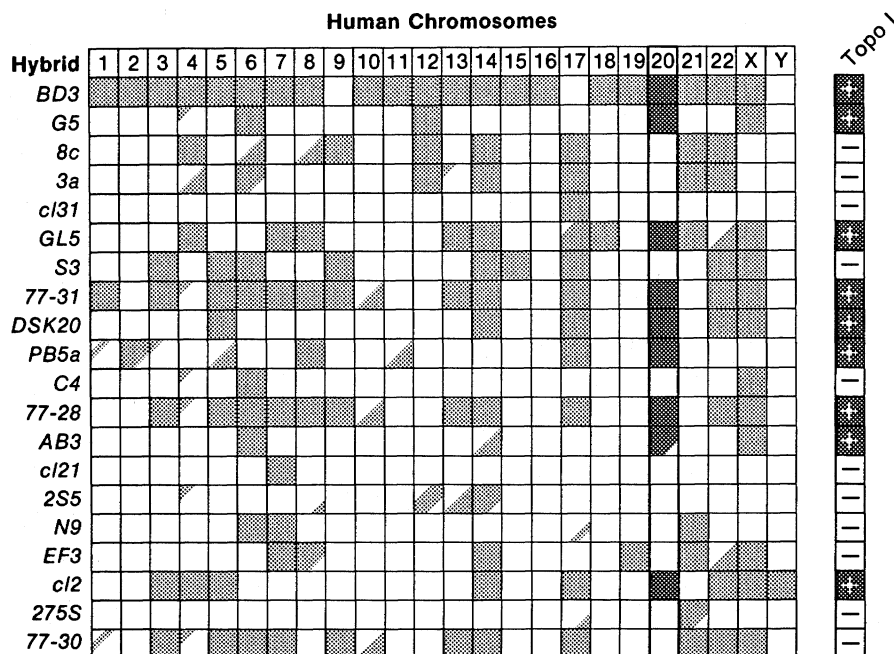


FIG. 3. Presence of the human *TOP1* gene in a panel of 20 rodent–human hybrids; \blacksquare indicates that the hybrid named in the left column contains the chromosome indicated in the upper row; \blacksquare indicates presence of the long arm (or a part of the long arm, indicated by a smaller fraction of stippling) of the chromosome shown above the column; \square indicates presence of the short arm (or partial short arm) of the chromosome listed above the column; \square indicates the absence of the chromosome listed above the column. The pattern of retention of the *TOP1* gene (Topo I) in the hybrid is shown in the column to the right of the figure where the presence of the gene in the hybrid is indicated by a stippled box with a plus sign and the absence of the gene is indicated by an open box enclosing a minus sign. The column for chromosome 20 is boldly outlined and stippled to highlight correlation of presence of this chromosome (or region of this chromosome) with the presence of the *TOP1* gene.

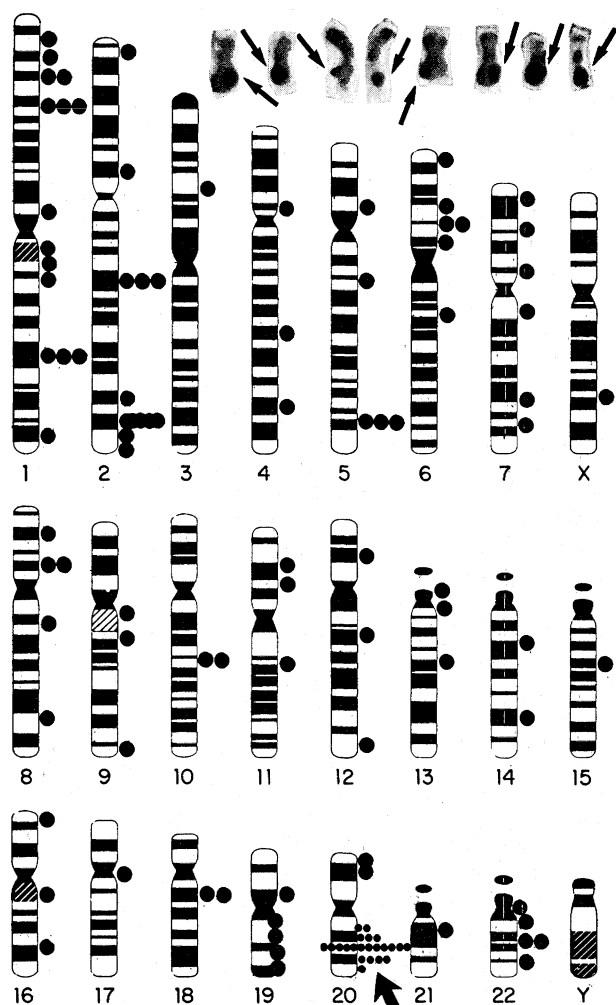


FIG. 4. *In situ* hybridization of the ^3H -labeled D1 probe of the human *TOP1* gene to human metaphase chromosomes. The solid circles next to the schematic representation of fluorodeoxyuridine-synchronized Wright-stained metaphase chromosomes represent all chromosomal grains observed. In the upper right corner, eight individual number 20 chromosomes from seven different metaphases are shown. Arrows indicate the grains in the q12-13.2 region.

spectrum of experimental tumors have been well-documented (35) and the drug is known to trap an abortive topoisomerase I-DNA covalent intermediate, termed the cleavable complex (21, 22), little is known about the chemical nature of the camptothecin-topoisomerase-DNA ternary complex. Cloning of the human DNA topoisomerase I gene is likely to accelerate studies on how camptothecin interferes with catalysis by DNA topoisomerase I and to facilitate the development of more effective topoisomerase I-targeting anticancer drugs.

The skillful technical assistance of Esther August is gratefully acknowledged. This work was supported by grants from the U.S. Public Health Services and the American Cancer Society. C.-C.J. was supported by a predoctoral fellowship from the National Science Council, Republic of China. Part of this work was carried out during the year 1986-87 when J.W.-P., J.C.W., and L.F.L. were in residence at the Institute of Molecular Biology of Academia Sinica; the support of Academia Sinica and that of the Guggenheim Foundation in the form of a fellowship to J.C.W. are also gratefully acknowledged.

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